

JOURNAL OF ANIMAL SCIENCE

The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Technical note: Comparison between two tracing methods with ultrasonography to determine lumen area of the caudal artery in beef cattle

G. E. Aiken, J. L. Klotz, B. H. Kirch, J. R. Strickland and D. L. Boykin

J Anim Sci 2009.87:371-374.

doi: 10.2527/jas.2008-1087 originally published online Sep 12, 2008;

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://jas.fass.org/cgi/content/full/87/1/371>



American Society of Animal Science

www.asas.org

Technical note: Comparison between two tracing methods with ultrasonography to determine lumen area of the caudal artery in beef cattle¹

G. E. Aiken,^{*2} J. L. Klotz,^{*} B. H. Kirch,^{*} J. R. Strickland,^{*} and D. L. Boykin[†]

^{*}USDA-ARS, Forage-Animal Production Research Unit, N220 Ag Science North, University of Kentucky, Lexington 40546; and [†]USDA-ARS, Jamie Whitten Delta States Research Center, 141 Experiment Station Rd., Stoneville, MS 38776

ABSTRACT: Two experiments were conducted to compare variation between 2 tracing methods in measuring cross-sectional lumen area of the caudal artery in 5 beef heifers on 3 different dates (Exp. 1) and to compare tracing methods in detecting changes in artery lumen area after 5 heifers were switched from a diet containing nonendophyte-infected tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort] seed to one containing endophyte-infected tall fescue seed (Exp. 2). Lumen area determined by tracing the Doppler flow signal was 25% less than that determined by tracing the intima of the connective tissue, but there was no difference ($P =$

0.90) in the variation of measures between the 2 methods in Exp. 1. Declines in lumen area were detected at the same level of significance ($P < 0.01$) for both tracing methods after cattle in Exp. 2 were switched from noninfected to infected tall fescue diets. Variation in lumen areas was different between noninfected and infected diets with tracing the Doppler flow signal ($P < 0.05$) or the intima of the connective tissue ($P < 0.01$). Results indicated that lumen area of the caudal artery can be measured with similar precision by tracing the intima of the connective tissue in the artery wall or the outer boundary of the Doppler flow signal.

Key words: artery area, caudal artery, Doppler ultrasonography, ergot alkaloid

©2009 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2009. 87:371–374
doi:10.2527/jas.2008-1087

INTRODUCTION

Doppler ultrasonography has been used to evaluate blood flow characteristics in dogs (Brown et al., 1991; Lee et al., 2004) and horses (Cipone et al., 1997; Raisis et al., 2000). The technology also has potential use as a noninvasive diagnostic or research tool in evaluating hemodynamics of cattle that are exposed to plants composed of toxic secondary metabolites.

Measurements of cross-sectional areas of vessels are needed to quantify blood flow rate and determine if vessels are in a constricted or relaxed state (Aiken et al., 2007). Ultrasound units and transducers used in human medicine and research have the resolution that is necessary to measure blood vessel diameter at the interface between the lumen and the endothelial intima (Poulin and Robbins, 1996; Shaw et al., 2007). Resolution of

ultrasound units and transducers that are often used by veterinary practitioners and researchers is generally insufficient to discern the endothelial intima in the blood vessel (Figure 1A, B). Therefore, lumen areas often are traced using either inner aspects of the elastin tissue layer at the interface between the adventitia-media layers in the artery wall (Cipone et al., 1997; Meinders and Hoeks, 2004) or the outer boundary of the flow signal obtained with Doppler color ultrasonography (Aiken et al., 2007). However, differences in precision between the 2 tracing methods are not known. A study was conducted with cattle to determine if there are differences in the variation of lumen area measurements of the caudal artery between tracing the interface between the adventitia-media layers or the outer boundary of the color Doppler flow signal methods, and if the 2 tracing methods have the precision needed to detect a vasoconstriction response to ergot alkaloids.

MATERIALS AND METHODS

Two experiments were conducted with crossbred (Angus × Brangus) heifers (12 to 14 mo) that were on diets devoid of ergot alkaloids from birth until the beginning

¹Mention of trade names or commercial products in the article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

²Corresponding author: glen.aiken@ars.usda.gov

Received April 5, 2008.

Accepted August 27, 2008.

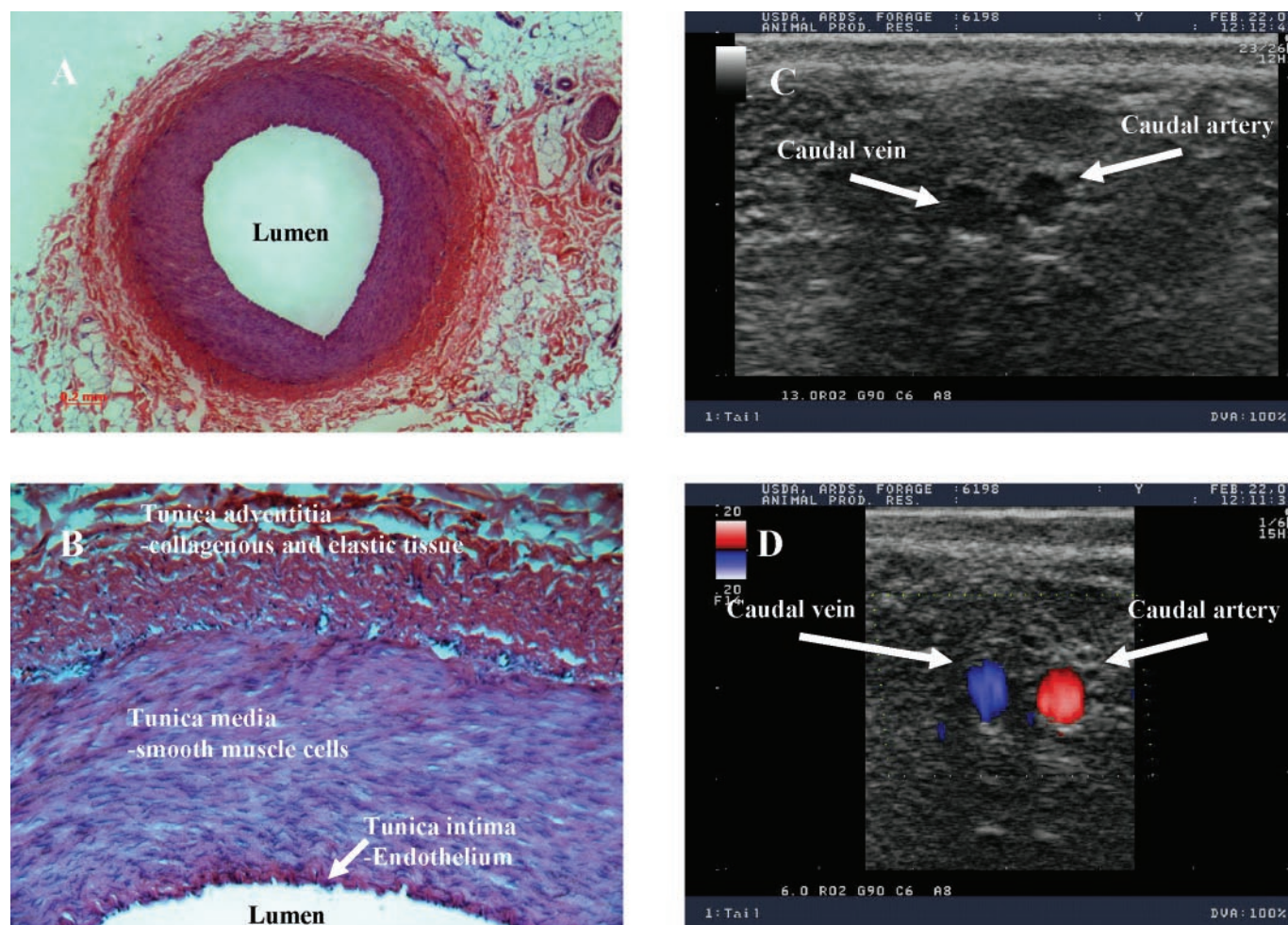


Figure 1. Cross-sections of a bovine caudal artery for hematoxylin-eosin stained tissues at 25 \times magnification (panel A) and 100 \times magnification (panel B) to demonstrate the tunica intima, tunica media, and tunica adventitia in greater detail, and for ultrasound scans taken in B-mode (panel C) and color Doppler (panel D). Caudal artery and surrounding connective tissue for histological cross-section evaluation was collected immediately after slaughter from a steer that was not used in the experiments. The section of artery was fixed in 10% buffered formalin, dehydrated using ethanol, cleared using xylene, and embedded in paraffin. Histological preparation of caudal artery samples was conducted utilizing hematoxylin-eosin staining procedures described by Allen (1992).

of the experiment. The experimental protocol was reviewed and accepted by the Institutional Animal Care and Use Committee at the University of Kentucky.

Experiment 1

Five crossbred Angus \times Brangus heifers [mean initial BW = 293 \pm 35 (SD) kg] were maintained on free-choice corn silage-concentrate rations and ultrasonically scanned on 3 dates (Feb. 20, Feb. 22, and Mar. 2, 2007). Cross-sectional ultrasound scans of the caudal artery at the 4th coccygeal vertebrae were taken using an Aloka 3500 Ultrasound Unit (Aloka Inc., Wallingford, CT) with a UST-5542 linear array (13 MHz) transducer set to a 2-cm depth. Ten B-mode images (Figure 1C) and 10 Doppler flow images (Figure 1D) were taken to determine cross-sectional artery lumen area. Overall gain was set at 90 and the flow gain was set at 14; the maximum gain before noise became apparent. A pulse frequency of 6 MHz was used for B-mode and Doppler flow images. Sample volume was set at 0.5 mm, which

sets the size of the pulsed-wave Doppler region to be examined. Following the freezing of an individual flow image, frames stored in the cine memory of the unit (42 frames with a 15-Hz frame rate) were searched to store the image exhibiting the maximum flow signal and assumed to be at peak systolic phase. For B-mode scans, the inner aspect of the acoustic reflection from the elastin connective tissue in the artery wall was used to trace for estimation of lumen area. For color Doppler images, the outer aspect of the flow signal in the artery was traced for estimation of lumen area.

For each tracing method, individual measurements of lumen area from 10 images were taken for 5 heifers on 3 dates. Comparisons between tracing method variances and means were made. For comparing tracing method means, the experimental design was a split plot where the main unit had a randomized complete block design with 2 tracing method treatments and 5 heifers as a block effect; the subunit was a repeated measure over 3 dates, and multiple images on each heifer \times tracing method \times date were subsamples. Tracing method

means were compared based on ANOVA for this design.

Variances of lumen area measurements for each tracing method, without assuming common variance between tracing methods, consisted of variance between images plus variance between dates and heifers partitioned into 3 components for date, heifer, and date \times heifer. This variance was expressed in the following equation:

$$V(Y_{ijkl}) = \sigma^2_{ij} + \sigma^2_{ik} + \sigma^2_{ijk} + \sigma^2_{l(ijk)}, \text{ for each } i, [1]$$

where i = tracing method, j = heifer, k = date, l = image.

The model used to compare variances was different from the model used to compare means because 1) heifer as a block effect can only be removed from error for mean comparison purposes given an assumption of common variance between treatment effects being compared and 2) the effect of date was considered a random source of error. Variance components, defined above, were analyzed using mixed models (SAS Inst. Inc., Cary, NC). A model allowing different variances for each tracing method was compared with a model assuming equal variances between tracing method with a likelihood ratio test (Littell et al., 2006). Statistical differences were determined using the $\alpha = 0.10$ level of significance.

Experiment 2

Five crossbred Angus \times Brangus heifers [mean initial BW = 346 ± 20 (SD) kg] were placed in individual pens and fed a mixture of chopped alfalfa hay (54% DM) and a concentrate (46% DM) that contained ground soybean hulls (47.5% DM), mineral premix (1% DM), molasses (4% DM), and ground nonendophyte-infected tall fescue seed (47.5% DM) for 7 d. Following this 7-d period, the heifers were fed the same chopped alfalfa-concentrate mixture with the exception that the concentrate contained endophyte-infected seed instead of nonendophyte-infected seed. Before feeding, 12 samples of infected seed were assayed for ergovaline (mean = $3.67 \mu\text{g/g}$ of DM; CV = 2.1%) by HPLC fluorescence (Yates and Powell, 1988). Sensitivity of detection was $0.1 \mu\text{g/g}$ of DM. Quantity of endophyte-infected seed was formulated to provide $0.85 \mu\text{g}$ of ergovaline/g of DM in the total diet.

Cross-sectional ultrasound scans of the caudal artery at the 4th coccygeal vertebrae were taken on d 5 during the period when the diet containing nonendophyte-infected seed was fed to all heifers, and on d 8 during the period when the ration containing endophyte-infected seed was fed. Scanning and measurements followed the same procedures as Exp. 1, with the exception that 3 B-mode and 3 color Doppler flow scans were collected from each heifer during each of the 2 scanning sessions. Differences in cross-sectional lumen area and variation between nonendophyte-infected and endophyte-infected

rations were determined for each tracing method using the t -test procedure of SAS. Measurements were averaged within each heifer \times diet combination resulting in heifers being the experimental units. Statistical differences were determined using the $\alpha = 0.10$ level of significance.

RESULTS

Experiment 1

Mean cross-sectional lumen area was greater ($P = 0.009$) with tracing the intima of the connective tissue (9.6 mm^2) than with the Doppler flow signal (7.2 mm^2). Total variance was 2.41 for connective tissue and 3.65 for Doppler flow signal based on a likelihood ratio test comparing a model with separate variances components for each tracing method to a model with equal variances components for both tracing methods. Variation in measurements did not differ ($\chi^2 = 4.0$, df = 4, $P = 0.406$) between the 2 tracing methods. There was a correlation ($r = 0.44$, $P < 0.09$) between the tracing methods.

Experiment 2

Variation in lumen area was different for the noninfected and infected diets by tracing both the Doppler flow signal ($P < 0.01$) and the intima of the connective tissue ($P = 0.07$). Mean cross-sectional artery lumen areas were different between nonendophyte-infected and endophyte-infected diets when tracing connective tissue ($P = 0.066$) or the Doppler flow signal ($P = 0.052$). Switching from endophyte-free to endophyte-infected diets caused lumen area to decline from 6.3 ± 1.4 to $2.9 \pm 0.2 \text{ mm}^2$ when tracing the Doppler flow signal and from 8.9 ± 1.3 to $5.4 \pm 0.5 \text{ mm}^2$ when tracing the connective tissue.

DISCUSSION

Greater lumen area with tracing the connective tissue was expected because layers of smooth muscle and endothelial cells could cause slight overestimation of artery area. Conversely, tracing the Doppler flow signal could underestimate lumen area due to lack of detection of reduced velocity blood flow in the outer aspects of the lumen.

Variation in measurements for Exp. 1 was similar between the 2 tracing methods even though the sources of error between the methods are different. The observer is likely the major source of error in measuring lumen area by tracing the connective tissue. Although observer is a source of error in tracing the flow signal, as previously discussed, there likely are some inaccuracies in detection of blood flow in the outer aspects of the lumen where flow velocities are reduced.

The correlation coefficient was less than 0.50; however, ranges in areas for both methods were low and

could have reduced an ability to detect a close association between the 2 measurements (Cornell and Berger, 1987). Range in lumen areas using connective tissue to trace was 7.2 to 11.5 mm², and the range using the Doppler flow signal to trace was 4.1 to 9.4 mm².

Caudal arteries in Exp. 2 were determined to constrict following the switch from nonendophyte to endophyte-infected diets for both tracing methods. A vasoconstriction response to ergot alkaloids has been shown with the bovine caudal artery (Aiken et al., 2007) and the bovine saphenous vein (Klotz et al., 2007). However, artery lumen areas in the present experiment were more variable with the nonendophyte diet. Aiken et al. (2007) reported greater variation in caudal artery lumen areas and blood flows in heifers on a nonendophyte-infected diet than those on an endophyte-infected diet and concluded that caudal arteries in cattle on nonendophyte-infected diets are more responsive to changes in ambient temperature than those on the endophyte-infected diet. The precision in detecting differences in lumen area between the 2 diets could have been increased if more than 3 ultrasound scans had been collected; however, a previous study showed that 3 ultrasound measures per animal were sufficient to detect changes in blood flow for cattle that were switched from nonendophyte-infected to endophyte-infected tall fescue diets (Aiken et al., 2007).

Conclusions

Results indicated that changes in caudal artery lumen area can be detected with B-mode or color Doppler ultrasound tracing methods after the cattle undergo a vasoconstriction response. Either method can be used to measure lumen area with approximately the same precision.

LITERATURE CITED

- Aiken, G. E., B. H. Kirch, J. R. Strickland, L. P. Bush, M. L. Loofer, and F. N. Schrick. 2007. Hemodynamic responses of the caudal artery to toxic tall fescue in beef heifers. *J. Anim. Sci.* 85:2337–2345.
- Allen, T. C. 1992. Hematoxylin and eosin. Pages 53–58 in *AIFP Laboratory Methods in Histotechnology*, 1st ed. E. B. Prophet, B. Mills, J. B. Arrington, and L. H. Sobin, ed. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC.
- Brown, D. J., D. H. Knight, and R. R. King. 1991. Use of pulsed-wave Doppler echocardiogram to determine aortic and pulmonary velocity and flow variables in clinically normal dogs. *Am. J. Vet. Res.* 52:543–550.
- Cipone, M., M. Pietra, G. Gandini, A. Boari, C. Guglielmini, and M. Venturoli. 1997. Pulsed wave-doppler ultrasonographic evaluation of the common carotid artery in the resting horse: Physiologic data. *Vet. Radiol. Ultrasound* 38:200–206.
- Cornell, J. A., and R. D. Berger. 1987. Factors that influence the value of the coefficient of determination in simple linear and nonlinear regression models. *Phytopathology* 77:63–70.
- Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, B. C. Arrington, and J. R. Strickland. 2007. Ergovaline-induced vasoconstriction in an isolated bovine lateral saphenous vein bioassay. *J. Anim. Sci.* 85:2330–2336.
- Lee, K., C. Mincheol, Y. Junghee, and J. Juhyun. 2004. Spectral waveform analysis of major arteries in conscious dogs by Doppler ultrasonography. *Vet. Radiol. Ultrasound* 45:166–171.
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. Appendix A1.6.1 in *SAS for Mixed Models*, Second Edition. SAS Institute, Cary, NC.
- Meinders, J. M., and A. P. G. Hoeks. 2004. Simultaneous assessment of diameter and pressure waveforms in the carotid artery. *Ultrasound Med. Biol.* 30:147–154.
- Poulin, M. J., and P. A. Robbins. 1996. Indexes of flow and cross-sectional area of the middle cerebral artery using Doppler ultrasound during hypoxia and hypercapnia in humans. *Stroke* 27:2244–2250.
- Raisis, A. L., L. E. Young, H. B. Meire, P. M. Taylor, K. Walsh, and P. Lekeux. 2000. Variability of Doppler ultrasound measurements of hindlimb blood flow in conscious horses. *Equine Vet. J.* 32:125–132.
- Shaw, J. A., B. A. Kingwell, A. S. Walton, J. D. Cameron, P. Pillay, C. D. Gatzka, and A. M. Dart. 2007. Determinants of coronary artery compliance in subjects with and without angiographic coronary artery disease. *J. Am. Coll. Cardiol.* 39:1637–1643.
- Yates, S. G., and R. G. Powell. 1988. Analysis of ergopeptine alkaloids in endophyte-infected tall fescue. *J. Agric. Food Chem.* 36:337–340.

References

This article cites 11 articles, 3 of which you can access for free at:
<http://jas.fass.org/cgi/content/full/87/1/371#BIBL>